

# PHYLOGENETIC ANALYSIS OF HPNP REVEALS THE ORIGIN OF 2-METHYLHOPANOID PRODUCTION IN ALPHAPROTEOBACTERIA. J. N. Ricci<sup>1</sup>, A. J. Michel<sup>2</sup>, and D. K. Newman<sup>1,2,3</sup>,

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Hopanoids are bacterial steroid-like lipids that can be preserved in the rock record on billion-year timescales [1]. 2-Methylhopanoids are of particular interest to geobiologists because methylation is one of the few chemical modifications that remain after diagenesis and catagenesis. 2-methylhopanes, the molecular fossils of 2-methylhopanoids, are episodically enriched in the rock record [2], but we do not yet have a robust interpretation for their abundance patterns.

2-methylhopanes were once considered biomarkers of Cyanobacteria [3], but multiple lines of evidence suggest this interpretation is no longer valid [4-6]. To understand what these molecular fossils may be telling us about ancient environments and communities, a variety of approaches have been used to gain insight into the taxonomic sources and cellular functions of 2-methylhopanoids. Upon the identification of the enzyme responsible for C-2 hopanoid methylation, HpnP, homologs were found in modern Cyanobacteria, Alphaproteobacteria, and an Acidobacterium [5] and in diverse environments [6]. Hopanoids have been proposed to play a role in membrane integrity and resistance to various stresses, such as high temperature and extreme pH [7-13], and recently methylation was shown to increase membrane rigidity [14].

Despite the emerging picture of modern 2-methylhopanoids, better understanding of their evolution is needed to interpret the significance of episodic 2-methylhopane deposits. While 2-methylhopanoids originate from certain bacterial sources on Earth today, this does not necessarily mean related organisms produced them in the past. One way to constrain this uncertainty is to determine the likely ancestral source of 2-methylhopanoids by decoding the evolutionary history of the HpnP methylase. Previous attempts were inconclusive due to the paucity of HpnP sequences at the time [5]. Since then, we made a targeted effort to clone more diverse environmental *hpnP* sequences from various habitats [6], as diverse sequences are needed to root the HpnP family. In addition, many more *hpnP*-containing genomes have been deposited in publically-available databases in the past few years. We report that it is now possible to derive a conserved and robust HpnP evolutionary history.

We find that 2-methylhopanoids originated in a subset of the Alphaproteobacteria, most likely in the

last common ancestor of the Bradyrhizobiaceae, Beijerinckiaceae, Methylobacteriaceae, and Methylocystaceae families. This conclusion is statistically robust and reproducible in multiple trials varying the outgroup, trimming stringency, and ingroup dataset used to infer the evolution of this protein. The capacity for 2-methylhopanoid production was likely horizontally transferred from the Alphaproteobacteria into the Cyanobacteria after the Cyanobacteria's major divergences.

Given that early 2-methylhopane deposits could have been made solely by Alphaproteobacteria before the acquisition of *hpnP* by Cyanobacteria, and that Alphaproteobacteria are thought to be ancestrally aerobic, we infer that 2-methylhopanoids likely arose after the oxygenation of the atmosphere. This is consistent with the geologic record—the oldest syngenetic 2-methylhopanes occur after the rise of oxygen, 1.64 Ga, in the Barney Creek Formation [1]. Also, these results suggest that the ancestral function of 2-methylhopanoids was not related to oxygenic photosynthesis but to a trait present in ancient Alphaproteobacteria. It is possible that the original function of 2-methylhopanoids and the physiology of its ancestral producers mimics those seen in modern Alphaproteobacteria. Today we know that many HpnP-containing Alphaproteobacteria are anoxygenic phototrophs, diazotrophs, and can utilize methanol as a carbon source [6]. It is tempting to speculate that ancestral 2-methylhopanoids might have indirectly enabled them to perform one of these functions.

**References:** [1] Brocks J. J. et al. (2005) *Nature*, 437, 866–870. [2] Knoll A. H. et al. (2007) *AP*, 133–164. [3] Summons R. E. et al. (1999) *Nature*, 400, 554–557. [4] Rashby S. E. et al. (2007) *PNAS*, 104, 15099–15104. [5] Welander P. V. et al. (2010) *PNAS*, 107, 8537–8542. [6] Ricci J. N. et al. (2014) *ISME*, 8, 675–684. [7] Poralla K. et al. (1984) *FEMS Microbiol Lett*, 23, 253–256. [8] Kannenberg E. L. and Poralla K. (1999) *Naturwissenschaften*, 86, 168–176. [9] Welander P. V. et al. (2009) *J. Bact*, 191, 6145–6156. [10] Welander P. V. et al. (2012) *Geobiology*, 10, 163–177. [11] Doughty D. M. et al. (2009) *Geobiology*, 7, 524–532. [12] Doughty D. M. et al. (2011) *PNAS*, 108, E1045–E1051. [13] Kulkarni G. et al. (2013) *J. Bact*, 195, 2490–2498. [14] Wu C. H. et al. (2015) *eLife*, 4.